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**TITLE** Method for Producing Immunoglobulin  
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### ABSTRACT

**[Purpose]** The invention relates to a method for producing immunoglobulin and an immunoglobulin monomer by means of an immunoglobulin (antigen) obtained therefrom.

**[Constitution]** The object immunoglobulin is obtained by adding ethylenediaminetetraacetic acid to a concentration of 0.5-10 mM or glycine to a concentration of 50-500 mM to whey obtained by precipitation of casein at the isoelectric point (pH 4.5-4.6) or by treatment with an enzyme such as rennin, adjusting the pH of the whey to 6.0-6.5 with a sodium citrate solution, bringing this into contact with a cation exchange resin and using an ultrafiltration module having a separation limit of 50,000 or 100,000 daltons.

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## CLAIMS

1. A method for producing immunoglobulin characterized in that the object immunoglobulin is obtained by adding ethylenediaminetetraacetic acid to a concentration of 0.5-10 mM or glycine to a concentration of 50-500 mM to whey obtained by precipitation of casein at the isoelectric point (pH 4.5-4.6) or by treatment with an enzyme such as rennin, adjusting the pH of the whey to 6.0-6.5 with a sodium citrate solution, bringing this into contact with a cation exchange resin and using an ultrafiltration module having a separation limit of 50,000 or 100,000 daltons.
2. A method for producing an immunoglobulin monomer characterized by removing immunoglobulin polymer by bringing the immunoglobulin fraction obtained by using an ultrafiltration module having a separation limit of 100,000 daltons on whey obtained in claim 1 into contact with a cation exchange resin.
3. A method for producing an immunoglobulin monomer characterized by removing the immunoglobulin polymer by bringing the immunoglobulin obtained in claim 1 into contact with a cation exchange resin.

## DETAILED DESCRIPTION OF THE INVENTION

### Field of Industrial Application

The present invention relates to a method for producing immunoglobulin and immunoglobulin monomer by means of immunoglobulin obtained thereby.

### Conventional Art

Bovine colostrum contains immunoglobulins in amounts of 64.9 mg of IgG1, 22.2 mg of IgG, 3.5 mg of IgA and 8.7 mg of IgM per ml (Butler, J. E., ed. Butler, J. E., "The Ruminant Immune System", pp. 3-55, Plenum Press, New York, 1981), and attempts have been made to separate these Ig's and to use them actively have been made in various directions.

The main target among Ig's is the IgG fraction which is contained in the highest amount, common preparation methods including the combination of salting out, gel filtration,

ion exchange and the like, with affinity chromatography with protein A as the ligand being used when specificity is required. While cost increases due to these preparation methods can be absorbed if antibodies against special antigens are obtained and used for research or pharmaceuticals, the costs of products obtained by these preparation methods can make them impractical if they are to be used in foods or similar types of products.

Therefore, aside from the preparation methods described above, preparation methods using FeCl (Kuwata, T. et al., Elimination of  $\beta$ -lactoglobulin from whey to stimulate human milk protein, J. Food Sci., 50, 605-609, 1985), NaCl (Mailliart, P. et al., J. Food Sci., 53(3), 743, 1988) and ultrafiltration (Kirihara Osamu, "Separation and Use of Bovine Immunoglobulin", Dairy Science, Food Product Research, 39, A-301-A-305, 1990).

These methods all have the advantage of being very simple and holding down manufacturing costs, but are lacking in specificity, and particularly aside from the ultrafiltration (UF) method, bring about an extreme decrease in antibody titer. Additionally, although the UF method is basically fractionation by means of molecular weight differences and is an efficient method, milk whey contains proteins that tend to bond with various substances such as  $\beta$ -lactoglobulin,  $\alpha$ -lactoalbumin and bovine serum albumin.

In particular, the aforementioned proteins have a considerable tendency to bond to lipids, and  $\beta$ -lactoglobulin bonds strongly to lactoferrin which is present in whey (Ena, J. M. et al., Isolation of human lactoferrin by affinity chromatography using insolubilized bovine  $\beta$ -lactoglobulin, J. Chromato., 525, 442-446, 1990), so that the apparent molecular weight of these proteins in whey is extremely high, thus presenting an obstacle to fractionation by UF treatment.

Therefore, when producing IgG by a UF method, the purity is about 70-80% (Minami, Yoshiyuki et al., "Method for Concentrating Immunoglobulin in Milk" (JP-A S60-75433), Nobertokote et al., "Method for Producing Milk and/or Colostrum Immunoglobulin Solution" (JP-A S61-68429)), thus containing various contaminants and leaving much to desire in terms of purity. Additionally, in conventional methods, IgG is often present as a polymer, the titer being close to impossible to make stable.

### Problems to be Solved by the Invention

The present invention has the object of improving on the various obstacles and problems of the conventional art.

### Means for Solving the Problems

Here, the present invention proposes a method for producing immunoglobulin

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characterized in that the object immunoglobulin is obtained by adding ethylenediaminetetraacetic acid to a concentration of 0.5-10 mM or glycine to a concentration of 50-500 mM to whey obtained by precipitation of casein at the isoelectric point (pH 4.5-4.6) or by treatment with an enzyme such as rennin, adjusting the pH of the whey to 6.0-6.5 with a sodium citrate solution, bringing this into contact with a cation exchange resin and using an ultrafiltration module having a separation limit of 50,000 or 100,000 daltons, and further proposes a method for producing an immunoglobulin monomer characterized by removing immunoglobulin polymer by bringing the immunoglobulin fraction obtained by using an ultrafiltration module having a separation limit of 100,000 daltons on whey obtained by the above method into contact with a cation exchange resin, and method for producing an immunoglobulin monomer characterized by removing the immunoglobulin polymer by bringing the immunoglobulin obtained by the above method into contact with a cation exchange resin.

### Functions

Immunoglobulin is obtained and immunoglobulin monomer produced therefrom by the above-described methods.

### Examples

(1) Preparation of Bovine Colostrum Whey: The colostrum was collected 0-3 days after delivery from a cow sensitized with human IgE by conventional methods from 3 weeks prior to delivery. The fat was eliminated by a cream separator to obtain skim milk. An equal amount of deionized water was added thereto, after which was added EDTA to a concentration of 0.5-10 mM (preferably 1 mM) or glycine to a concentration of 50-500 mM (preferably 100 mM), and the result adjusted to a pH of 4.5-4.6 with citric acid. The precipitants were removed by centrifugal separation to obtain whey. The pH of this whey was adjusted to 6.0-6.5 with sodium hydroxide, after which it was ready for experimentation. Here, the antigen (human IgE) for sensitization was chosen simply for the purposes of explaining this invention.

(2) Preparation of IgG Monomer: 20 g of DEAE-cellulofine (product of Seikagaku Kogyo) were added to 300 ml of the whey obtained in (1) above, and the result was blended mildly, after which it was filtered with a Buchner funnel, the non-adsorbed fraction collected, and an ultrafiltration performed thereon using an ultrafiltration module (product of Asahi Kasei) having a separation limit of 50K or 100K daltons. The concentrated retentate was diluted with a 10 mM citrate buffer solution (pH 6.0-6.5) containing 1mM of EDTA or 100 mM of glycine, and ultrafiltration repeated. After removing the low molecular substances by this operation, the retentate was lyophilized.

(3) The whey obtained in (1) above was filtered in the ultrafiltration module used in the above (2) in the presence of a buffer solution containing 1 mM EDTA or 100 mM glycine, the resulting retentate was brought into contact with DEAE-cellulofine, the

non-adsorbed fraction was collected, and this was lyophilized.

(4) When preparing an IgG monomer by the method described in (2) above, the IgG sometimes forms polymers due to physical forces such as pressure and shear caused by pumps during ultrafiltration. These IgG polymers were removed by contact with DEAE-cellulofine as in the procedure described in (3), and the result was lyophilized.

Next, the yield, purity and titer of the IgG preparations obtained by the methods described in (2), (3) and (4) are shown together in Table 1.

TABLE 1

Proc.	Yield (%) <sup>1</sup>	Purity (%) <sup>2</sup>	Titer ( $\times 10^3$ /mg·solid)	Titer ( $\times 10^3$ /mg·protein)
(2)	84.3	90.2	3.1	3.2
(3)	67.6	88.5	2.9	3.1
(4)	80.1	93.3	3.2	3.3

1\*: The yield was calculated with the IgG content of the whey obtained by the method described in procedure (1) as 100%.

2\*: The purity was calculated as the IgG monomer amount by means of gel filtration or ELISA process.

### Effects

According to the above-described method, immunoglobulin can be obtained by adding ethylenediaminetetraacetic acid and glycine to whey, and using a cation exchange resin and ultrafiltration module at a pH of 6.0-6.5.